35 DAY, 60 FOOT AIR SATURATION DIVE WITH RATS: EFFECTS ON EEG AND VISUALLY EVOKED CORTICAL RESPONSE

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SUMMARY PAGE

THE PROBLEM

To determine whether a long-term exposure in hyperbaric air at a depth of 60 feet produces any changes in neural functioning, as measured by the electroencephalogram (EEG) and visually evoked cortical response (VER).

FINDINGS

EEG and VERs were recorded from chronically implanted rats before, during, and after a 35 day exposure to a depth of 60 feet. Significant changes occurred in both the EEG and VER, but these effects were not related to hyperbaric exposure since similar changes occurred in a control group which remained at the surface.

APPLICATION

The results imply that neural functioning was not impaired by the long-term exposure to a depth of 60 feet. This conclusion is relevant to shallow depth, human saturation exposures.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Work Unit MF51.524.004-9015DA5G. The present report is Number 10 on this work unit. It was submitted for review on 30 July 1973, approved for publication on 12 September 1973 and designated as NavSubMedRschLab Report No. 756.

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ABSTRACT

EEG and visually evoked cortical responses (VERs) were recorded from rats before, during, and after a 35 day exposure to hyperbaric air at a depth of 60 ft. Recordings were obtained from chronically implanted cortical electrodes each week during the course of the dive. Although systematic changes occurred in both the EEG and VER during the experiment, similar changes occurred in a control group which remained at the surface. Thus the hyperbaric saturation exposure produced no significant changes in the EEG or VER. This result implies that a long-term exposure to a depth of 60 feet does not impair the general functioning of the nervous system.

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INTRODUCTION

In view of the growing interest in long-term, shallow depth, saturation diving, it is important that the possibility of progressive physiological changes due to such long exposures be thoroughly investigated. To this end, NSMRL has completed two air saturation dives with rats in which a wide variety of physiological measures were monitored.[1,2] The first dive involved a 60 day exposure to a depth of 50 feet. The depth was increased to 60 feet in the second experiment which also had a planned duration of 60 days. Due to technical problems related to the air supply, however, the second dive was terminated after 35 days. In this second dive, EEG and visually evoked cortical responses (VERs) were recorded from a group of rats for the first time during a long-term exposure. This report is concerned with the results obtained for these two neuro-physiological measures during this 35 day, 60 foot saturation dive.

Subjects

A total of 13 male albino rats (Charles River CD strain) were used in the experiment. They weighed about 400 gms. at the time of surgery.

Surgery

The surgical and recording techniques were the same as described in detail in a previous report.[3] The rats were anesthetized with sodium pentobarbitol and mounted onto a standard stereotaxic instrument. A single midline incision was made and the dorsal surface of the skull exposed and rubbed clean. A small hole for the active electrode was hand-bored through the skull over the rat's primary visual cortex (3 mm lateral and 2 mm anterior to the lambda). Reference and ground electrode holes were placed near the ipsilateral and contralateral frontal sinuses, respectively (3 mm lateral and 5 mm anterior to the bregma). Three self-tapping stainless steel screws (size #00, 1/8 in. long) were screwed into the skull holes, providing tightly secured epidural electrodes. A miniature Amphenol connecting socket was then mounted on the dorsal surface of the skull. Leads from the socket were wrapped tightly around the screws, and the screws and socket assembly were embedded in a mound of dental acrylic. The incision was then sutured around the base of the hardened acrylic pedestal. A recovery period of one to two weeks elapsed before the start of the experiment.

Recording Technique

All recording was done with the rats placed in a small Bethlehem hyperbaric chamber. Two rats at a time were held in a special restraining box and connected to specially designed plugs. The EEG from each rat was amplified by a separate Grass P511 pre-amplifier and recorded on FM tape (Hewlett-Packard

Model 3000 recorder). EEG frequency analyses were later obtained using a Federal Scientific spectrum analyzer (Model UA-10A) and spectrum averager (Model 1010). The visual stimulation for VERs was provided by flashing a Grass PS-2 photo stimulator directly into the porthole of the chamber. The intensity of the photo stimulator was set at "4". The amplified EEG of each rat was connected to separate channels of a Computer of Averaged Transients (Technical Measurement Corp.) which enhances that portion of the ongoing EEG that is time-locked to the light flashes. Paper records of the averaged VERs were obtained by an X, Y recorder.

Procedure

The rats were assigned at random either an Experimental (E) or Control (C) group. The six E rats, along with a large number of non-implanted rats, spent 35 days in a large hyperbaric chamber at a depth of 60 feet. A life-support system maintained temperature, humidity, oxygen, and carbon dioxide at acceptable levels. Daily cage maintenance was performed by tenders who entered the chamber through an outer lock. The seven C rats were kept in the chamber room during the dive. Both groups of rats were individually housed in identical partitioned cages.

To record from the E rats during the dive, the small chamber was placed in the outer lock of the large chamber. The lock was pressurized to 60 ft, and two E rats at a time were placed in the restraining box, plugged in, and sealed inside the small chamber. The lock was then rapidly brought to the surface. The small chamber was removed and connected to an air supply to provide venting, while maintaining the 60 ft. depth. Continuous venting was also used for the C rats, but the chamber was not pressurized.

Pre-dive data were collected from all of the rats during the week prior to the start of the dive. The standard test session consisted of recording about 1.5 min. of EEG, obtaining a VER for a flash rate of 16 Hz, and then recording another 1.5 min. of EEG, followed by a VER for a 2 Hz flash rate. The VERs were based on the average of 100 one-second sweeps. Each test session for a pair of rats took less than 10 minutes.

After the pre-dive test session, data were collected 1, 2, 3, 4, and 5 weeks after the start of the dive. Each week all the rats were tested on the same day. The E rats were safely decompressed on day 36 of the dive. Post-dive data were obtained a few hours after decompression, and again on the following day.

RESULTS

The EEG and VER data were analyzed for only five rats in each group. The implanted socket on one of the E rats broke off during the dive, and the results for the last two C rats were not used in order to simplify statistical comparisons between groups.

EEG

Averaged frequency spectra were obtained for the recorded EEG of each rat

for each test session. Each analysis was based on the average of 16 frequency spectra for successive 4 second EEG epochs. Separate analyses were obtained for each phase of the test session: the two recordings of ongoing EEG, and the EEG during the VER flash rates of 16 Hz and 2 Hz. A typical set of averaged frequency spectra is shown in Fig. 1. The analysis of the ongoing EEG of the rat generally shows a peak in the 5-8 Hz range and also activity in the 2-4 Hz range. The spectrum for EEG during the 16 Hz flash has a peak at 16 Hz, as well as harmonics at higher frequencies. For the 2 Hz flash, peaks generally occur at about 2, 4, and 6 Hz. The amplitude data for each of these spectral components were subjected to analyses of variance. These statistical analyses revealed that neither the ongoing 5-8 Hz activity nor the peak at 16 Hz showed any significant changes during the dive for either group of rats (p's $>.05$). The 5-8 Hz activity during the 16 Hz flash did change over successive tests ($p < .01$), but these changes were not systematic. The 2-4 Hz activity (both ongoing and during the 16 Hz flash) showed a significant tendency to increase over the testing weeks (p's $<$ .05), and significant changes also oc-

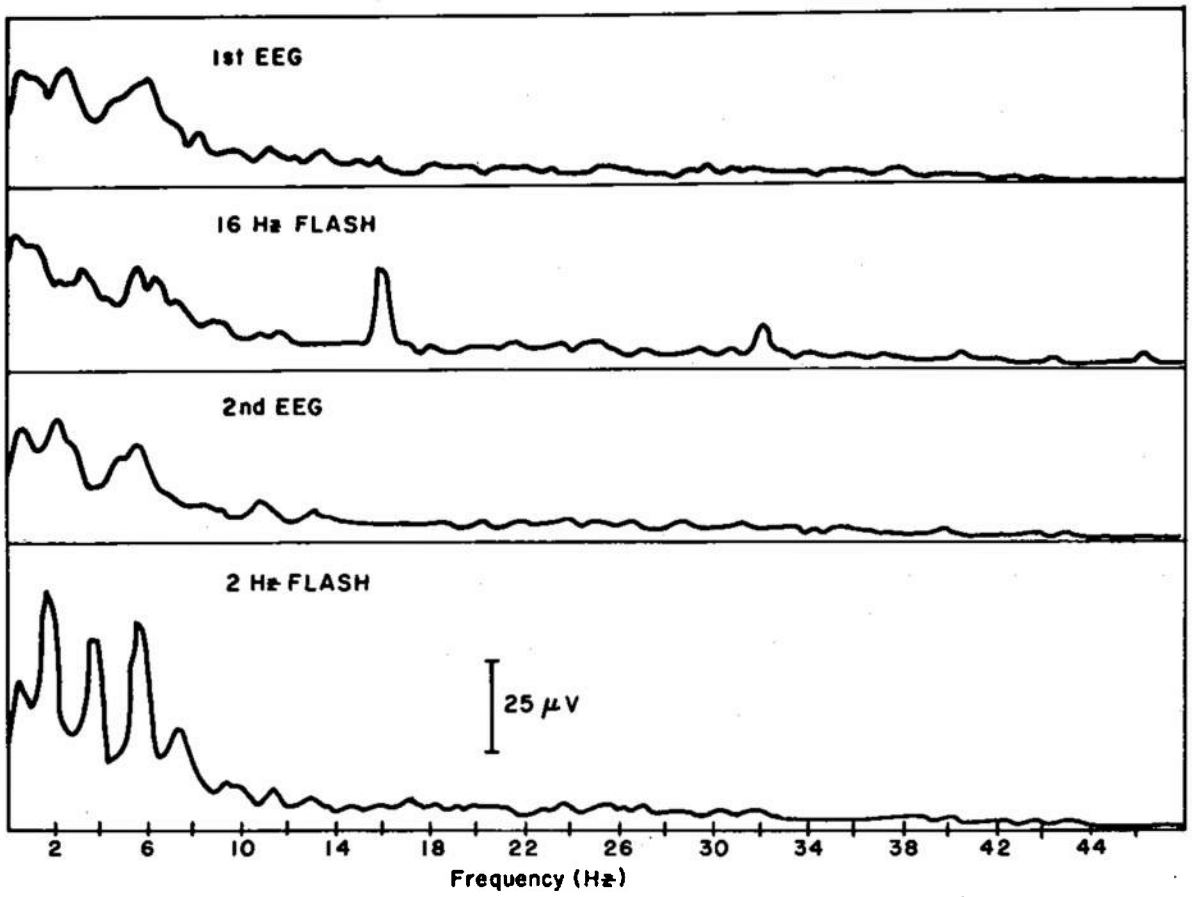

Fig. 1. Typical set of averaged EEG frequency analyses. These records represent the average of 16 individual analyses of successive 4 sec EEG epochs (64 sec. of EEG).

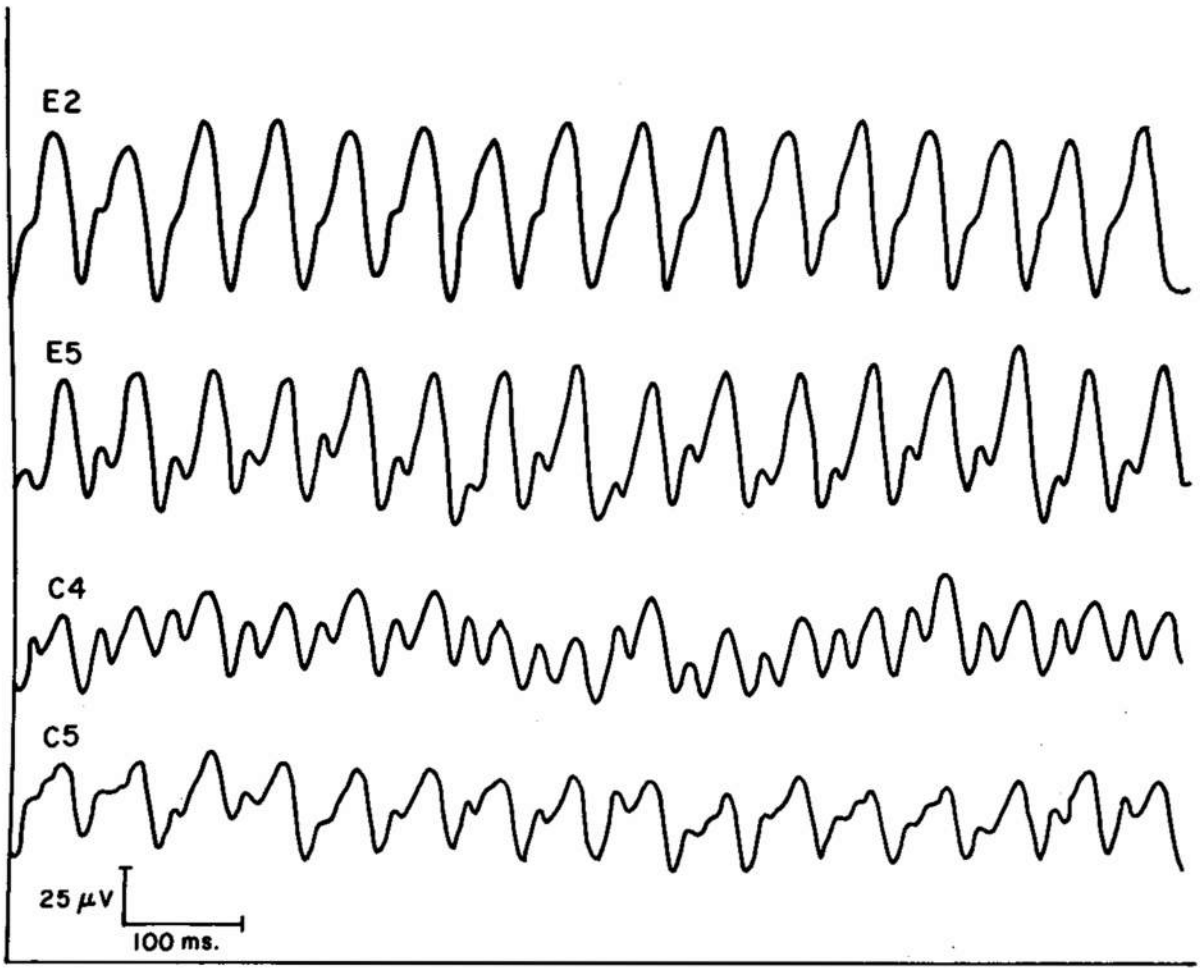

Fig. 2. Examples of VERs elicited by a photo stimulator flash rate of 16 Hz. The sweep time for the entire record is 1 sec.

curred in the 2 Hz and 4 Hz activity during the 2 Hz flash (p's <.05). However, there were no significant Group x Test interactions (p's >.05) for the EEG components which changed over time; that is, the changes were similar for both the E and C rats. Thus, none of the EEG changes were related to the long-term hyperbaric exposure.

VERs

Typical VERs elicited by a photo stimulator flash rate of 16 Hz are shown in Fig. 2. At 16 Hz the VER consists of 16 fairly regular, sinusoidal responses (often a double response consisting of 32 peaks). These records were analyzed by measuring the peak to peak amplitudes of each of the 16 responses, and computing the mean and standard deviation for each record. A Z score (mean/standard deviation) was also computed to provide a variability measure which is independent of amplitude. Analyses of variance were carried out for both the mean amplitudes and the Z scores. There were no significant differences between the E and C

groups, between the different testing weeks, or for the Group X Test interactions (p's >.10). Thus the 36 day exposure to a 60 foot depth had no effect on the VERs elicited by the 16 Hz flash.

Some changes over time did occur for the VERs elicited by a 2 Hz flash. Examples of VERs at 2 Hz are shown in Fig. 3. The main components are labelled a - f on the top record. The latencies of each component were measured, and the amplitudes, defined as the peak to peak deflection from the previous component, were determined. Analyses of variance for each component showed that components a, c and d tended to decrease in amplitude over the eight tests (p's <.01), and component e increased in amplitude (p <.01). The latencies of the late components d, e and f significantly decreased over time (p <.01). However, since there were no Group x Test interactions which were either statistically significant or meaningful, all of the changes occurring over time were similar for both the E and C groups. Thus the changes were not related to exposure to hyperbaric air.

There was one change in the 2 Hz VER which may have been related to the dive. For four of the six rats in the E group, there was at least some increase

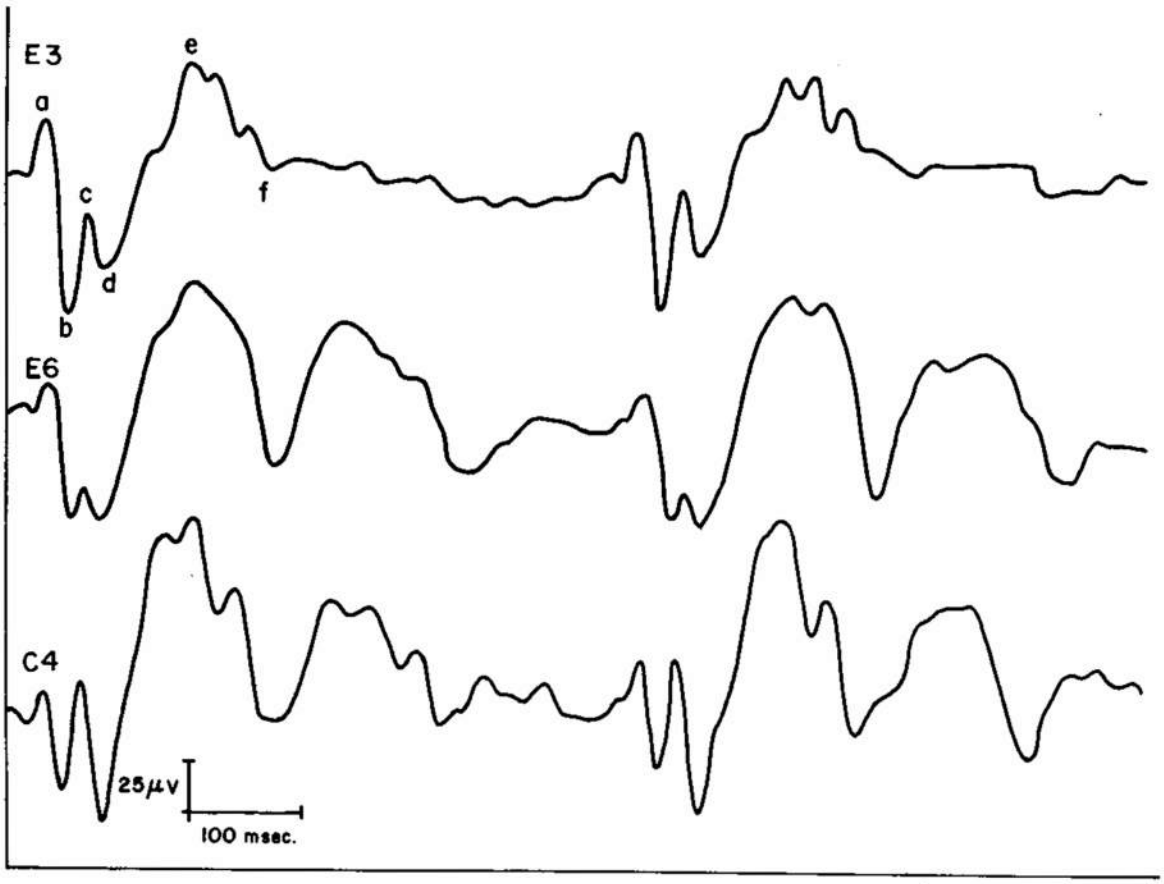

Fig. 3. Typical VERs elicited by a flash rate of 2 Hz. The major components are labelled a-f on the top record.

in the amplitude of the repetitive after-discharge which follows the response to the flash. Normally, little or no after-discharge occurs in an awake rat. This repetitive activity tended to increase for the E rats as the dive progressed, but this tendency was not entirely consistent. The activity did not occur during the post-dive tests, and no increase in after discharge occurred for any of the C rats. It is not certain, however, whether the activity was a true after-discharge, or whether it was a general repetitive activity which was superimposed on the entire record.

Relation Between EEG and VER

Since the VERs and the frequency analyses for EEG during the 16 Hz and 2 Hz flashes are derived from the same EEG input, there should be similarities in the results based on these two methods of analysis. In general, there was in fact a good correspondence between the EEG and VER results. Both analysis methods indicated no changes in the response to the 16 Hz flash. Similarly, both methods revealed changes over time in the 2 Hz response which were not related to hyperbaric exposure. In more objective terms, there should also be similarities between the actual amplitudes of the VERs and the amplitudes of the spectral peaks at 16 Hz and 2 Hz. At 16 Hz there was a good amplitude correspondence. Although the amplitude of the spectral peak at 16 Hz was consistently less than the mean amplitude of the 16 Hz VER, the correlation coefficients for the EEG and VER amplitudes of the individual rats were generally quite high, ranging from .42 to .90 for nine of the 12 rats. Averaging across the eight test sessions, the correlation between the mean amplitudes for the 12 rats was .83.

The amplitude correlations for the 2 Hz flash were quite low. The range was .27 to -.13 for 11 of the 12 rats. Averaging across test sessions, the correlation for the mean amplitudes of the individual rats was -.56. Correlations were also computed using the spectral peaks at 4 Hz and 6 Hz instead of the 2 Hz amplitudes. There were again no consistently high coefficients. Thus there was essentially no relationship between the spectral and VER amplitudes for the 2 Hz flash.

DISCUSSION

The results of this experiment indicate that the exposure of rats to a simulated depth of 60 feet for 35 days produces no significant changes either in the EEG or in VERs elicited by the fast or slow flash rates. To the extent that the EEG and VER are correlates of the state of the nervous system, this lack of effect demonstrates that neural functioning was not adversely affected by the hyperbaric exposure.

The one change which may have been related to the hyperbaric exposure was the occurrence of repetitive activity during the 2 Hz flash for the E rats. It should be emphasized, however, that the true cause of this repetitive activity is not known. The discharge was not evident in the EEG frequency analyses, suggesting the possible involvement of a time-locked noise artifact. Another possible cause of the repetitive activity was a temporary CO_2 build-up or O_2

depletion inside the small chamber during the period before the sealed chamber was connected to the air supply. However, it is quite possible that the venting during the recording session quickly eliminated any changes due to a non-venting period. Thus further research is necessary to clarify whether the repetitive activity might be due to an artifact or to the CO_2 build-up, or to slight oxygen toxicity or nitrogen narcosis.

The occurrence of systematic changes in the EEG and 2 Hz VER for both groups of rats during the experiment has both practical and theoretical implications. On the practical side, it is obvious that changes may occur in electrophysiological measures merely as a function of repetitive testing. Thus an adequate control group is essential to separate experimental effects from repetitive testing effects. If a control group had not been used in the present experiment, erroneous conclusions would certainly have been made concerning the effects of long-term hyperbaric exposure.

The VER changes which occurred during the experiment for both the E and C rats are of theoretical interest because the changes may represent long-term habituation to the photic stimulus. Habituation of the VER in the rat has recently been demonstrated.[4] When ten successive VERs were obtained, with 90 sec. elapsing between each trial, the early components of the VER did not change, but later components gradually decreased in amplitude while other components increased. Similar changes occurred in the present experiment, but with a full week elapsing between trials.

REFERENCES

1. Murray, R. D., Stewart, S. D. Heyder, Eily and Adams, G. M. Animal saturation diving at 50 and 60 ft. Naval Submarine Medical Research Laboratory, Groton, Conn. Rep. No. ___ 1973 (in press).

2. Heavers, B. A., Messier, A. A., Montanti, R. and Schaefer, K. E. CO_2 effects on acid-base balance in air saturation dives. Naval Submarine Medical Research Laboratory, Groton, Conn. Rep. No. 742, April 1973.

3. Ferris, S. H. and Bartus, R. T. A method for recording and analyzing EEGs and VERs from rats under hyperbaric conditions. Naval Submarine Medical Research Laboratory, Groton, Conn. Rep. No. 747, July 1973.

4. Bartus, R. T. and Ferris, S. H. Habituation and dark adaptation effects on VERs in rats. Naval Submarine Medical Research Laboratory, Groton, Conn. Report, (in press).

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EEG and visually evoked cortical responses (VERs) were recorded from rats before, during and after a 35 day exposure to hyperbaric air at a depth of 60 feet. Recordings were obtained from chronically implanted cortical electrodes each week during the course of the dive. Although systematic changes occurred in both the EEG and VER during the experiment, similar changes occurred in a control group which remained at the surface. Thus the hyperbaric saturation exposure produced no significant changes in the EEG or VER. This result implies that long-term exposure to a depth of 60 feet does not impair the general functioning of the nervous system.

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